Isolation and Characterization of Two Antibiotic-Producing Bacteria

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Abstract

The discovery of antibiotics with novel mechanisms has plateaued in the last twenty years. As antibiotics are continually used in modern society, bacteria are becoming increasingly resistant to the existing classes of antibiotics. A group of pathogens, the ESKAPE pathogens, are particularly dangerous, as they are resistant to most known classes of antibiotics; novel classes of antibiotics are desperately needed. Two bacteria capable of producing antibiotics against ESKAPE pathogen *Staphylococcus aureus* and *Escherichia coli* (in place of ESKAPE pathogen *Enterococcus faecium*) were isolated from soil, analyzed using microscopy, and subjected to various physiological tests. These tests yielded that both bacteria are motile, gram positive, spore-producing, rod-shaped aerobes capable of producing biofilm. The purification and PCR of the 16s rRNA gene from these bacteria was unsuccessful, but results of previously mentioned tests suggest that it is likely in the genus *Bacillus*. Though several antibiotics currently in use are produced by *Bacillus* species, further investigation of the mechanism of these antibiotics could be a potential avenue for future pharmaceutical research.

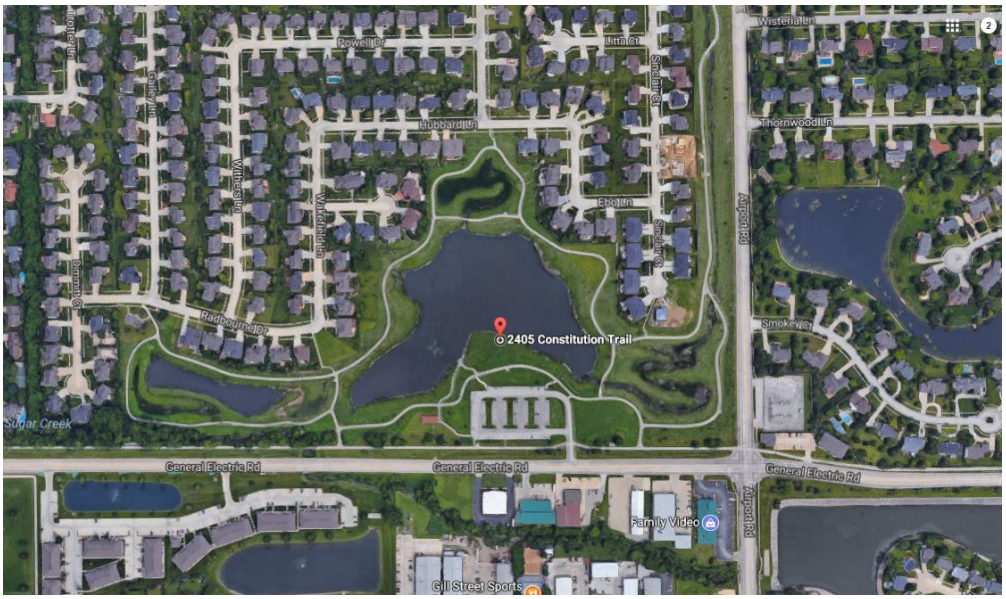

Figure 1. Location of soil sample for antibiotic testing. A sample of soil was collected at 6:40 pm on 8/29/2017 at 40.503597° N, -88.926748° W. Sample was taken from 4 cm below ground. Weather at the time of collection was sunny, with a temperature of 24.5° C. Sample was stored at room temperature until plating. Red pin shows location of sample collection.

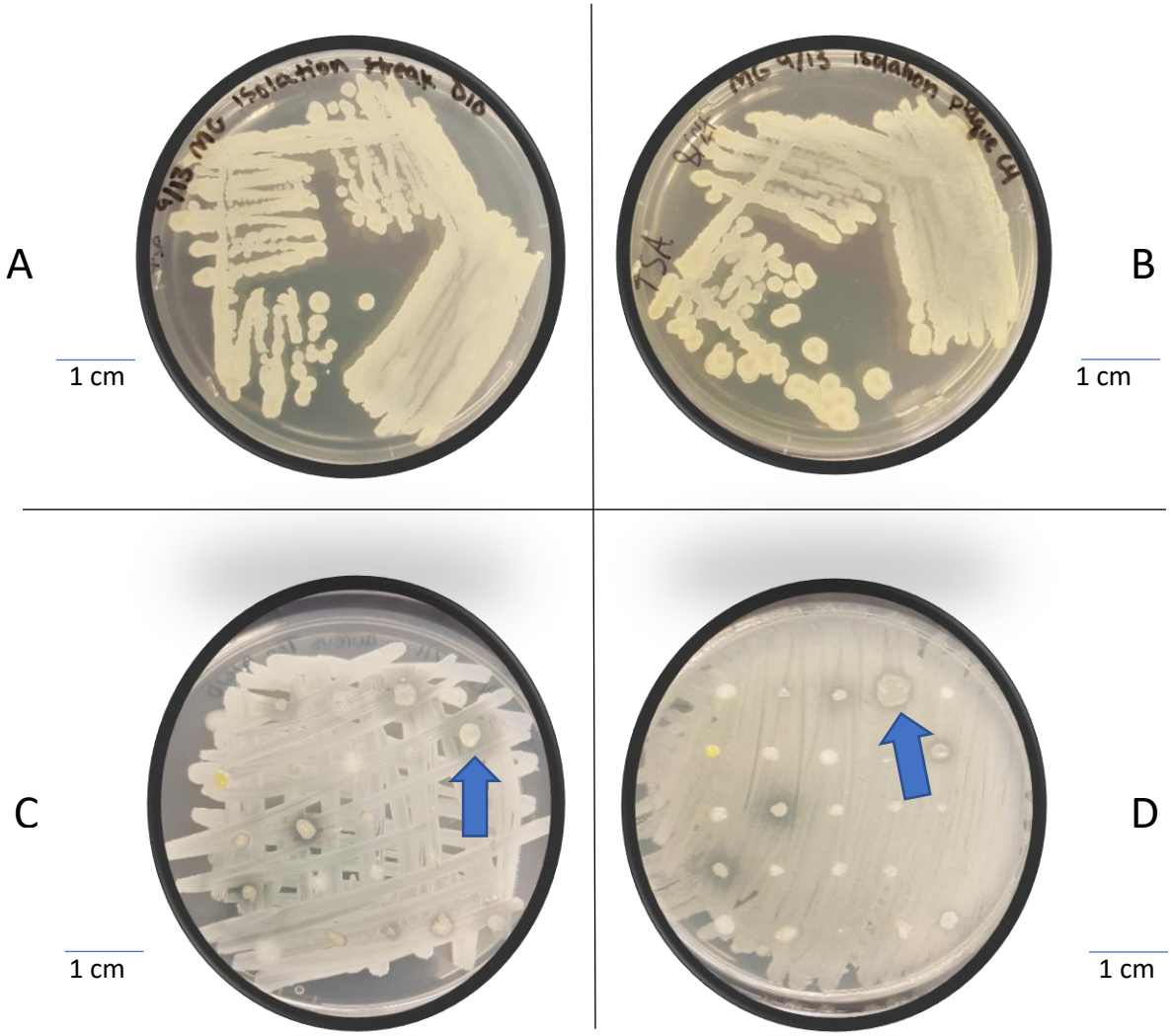

Figure 2. Isolation and morphology of two bacterial colonies with zones of inhibition against *Staphylococcus aureus* and *Escherichia coli*. Fifty bacterial colonies were patched to TSA plates coated with lawn of *S. aureus* or *E. coli*. Plates were incubated at 25°C, and monitored for the appearance of zones of inhibition. Two colonies with zones of inhibition were chosen for further characterization. Colony D10 (Picture C, blue arrow) exhibited a zone of inhibition against *S. aureus* extending approximately 2 mm around the colony. Colony C4 (Picture D, blue arrow), produced a 0.7 mm zone of inhibition against *E. coli*. Both D10 and C4 were streaked for isolation on TSA plates and incubated at 25°C for two days. D10 produced circular, cream-colored, pulvinate colonies of 2 mm in diameter with a glistening appearance (Picture A). C4 produced circular, cream-colored, umbonate colonies of 4 mm diameter, with a duller appearance (Picture B).

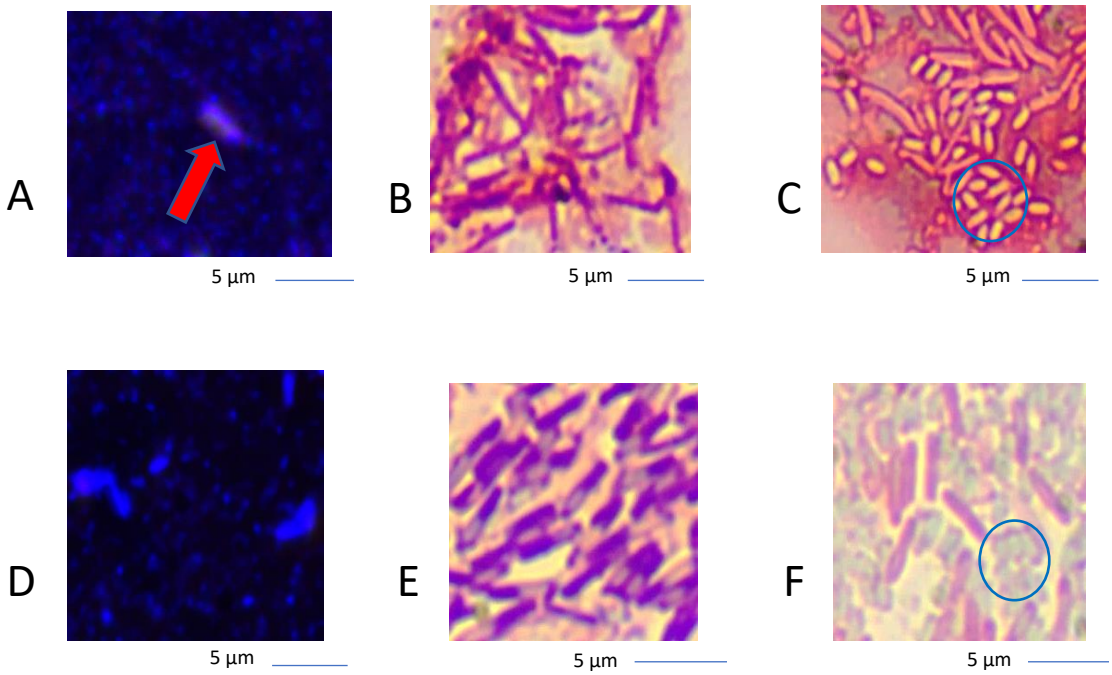

Figure 3. Capsule stain, gram stain, and endospore stain of two bacterial samples capable of producing an antibiotic against both *Staphylococcus aureus* and *Escherichia coli*. Bacteria C4 and D10 were cultured on individual Tryptic Soy Agar (TSA) plates at room temperature until staining. Images of capsule stain, gram stain, and endospore stain were taken on a light microscope at 100X magnification. Neither C4 or D10 showed evidence of a capsule under the specific conditions and timeframe of this stain. Picture A shows a C4 cell with no halo around it, while Picture D shows light spots not associated with cells. The gram stain showed that C4 is a rod-shaped gram positive bacteria of about 3 microns in length (Picture B), and D10 is a gram positive, rod-shaped bacteria of approximately 2.5 microns (Picture E). The endospore stain showed that both C4 and D10 are capable of producing endospores (Pictures C and F, respectively). Blue circles indicate clusters of endospores. Red arrow indicates bacterial cell on capsule stain.

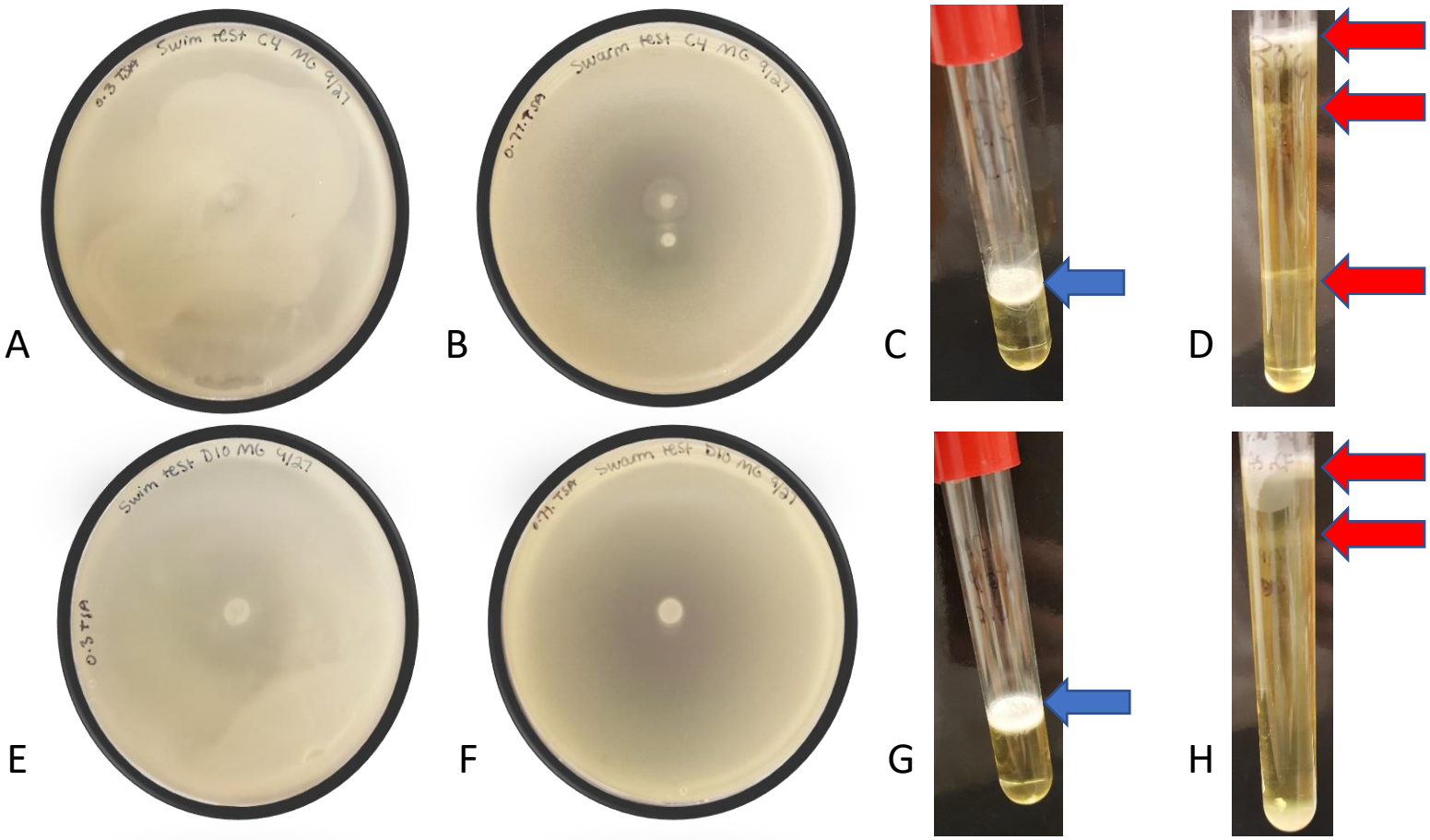

Figure 4. Physiological characterization of two bacterial samples capable of producing antibiotics against *Staphylococcus aureus and Escherichia coli.* Samples C4 and D10 were tested for their ability to swim and swarm, biofilm production, and oxygen tolerance. To determine if C4 and D10 are capable of swimming and swarming, colonies were resuspended in tryptic soy broth, and spotted onto a TSA plate with an agar concentration of 0.3% (for swim test) and 0.7% (for swarm test). To determine if C4 and D10 produce biofilms, tryptic soy broth was inoculated with the respective bacteria. Finally, to examine the oxygen tolerance of C4 and D10, thioglycolate medium was inoculated with the respective bacteria. All tests were incubated at room temperature for two days. As evidenced by the spread of the colonies, both C4 and D10 are capable of swimming (Pictures A, E) and swarming (Pictures B, F). Both C4 and D10 are also capable of producing biofilms, as evidenced by the presence of a pellicle (Pictures C, G). Based on the oxygen tolerance test, C4 and D10 are likely obligate aerobes. C4 grew in 3 bands, the largest of which was at the top of the tube (Picture D). D10 produced 2 bands, and the top one was significantly larger (Picture H). Blue arrows indicate pellicles. Red arrows indicate cell growth in oxygen tolerance test.

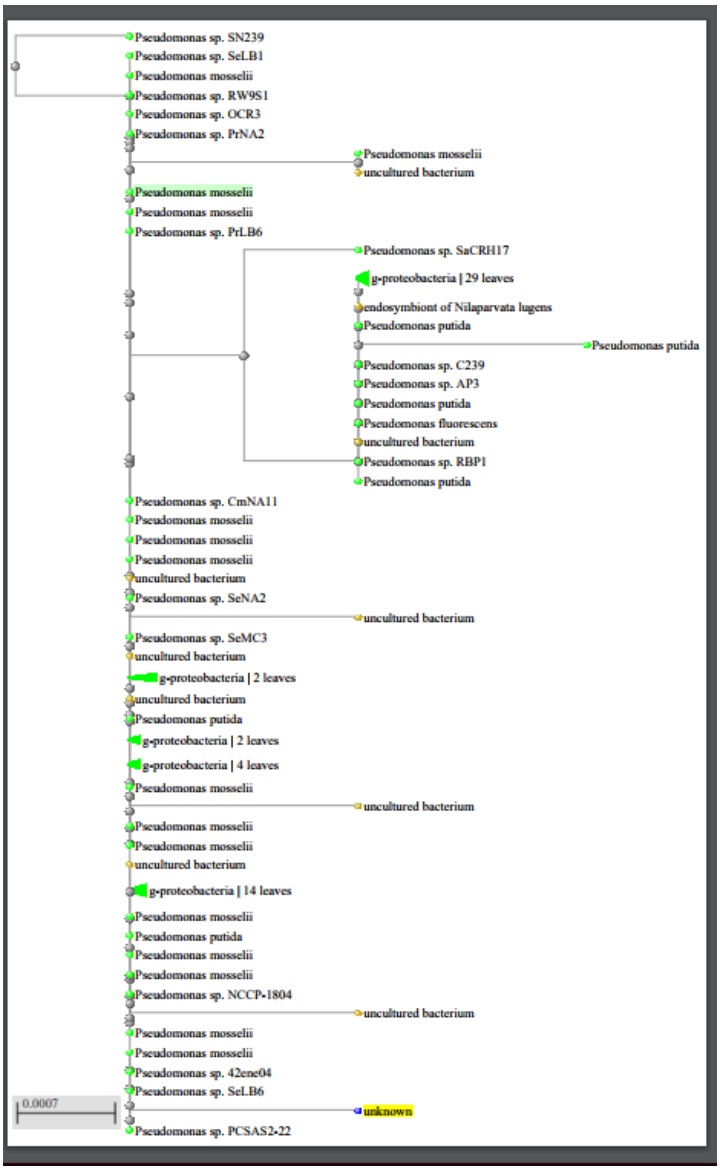

Figure 5. Phylogenetic tree of an unknown soil bacterium capable of producing an antibiotic against *Staphylococcus aureus*. Genomic DNA was purified from a bacterial culture on a TSA plate, and the 16s rRNA gene was amplified using PCR. The product of PCR was sent to Eurofins Genomics for Sanger sequencing. Upon receiving the sequence, the 16s rRNA gene was analyzed using SnapGene and compared to known sequences using NCBI Nucleotide BLAST. The nucleotide sequence of the unknown bacteria's 16s rRNA gene shares 99% identity with *Pseudomonas putida* and *Pseudomonas mosselli*, with an E value of 0.0 for both species. Yellow highlight indicates position of unknown bacteria on phylogenetic tree.